App. No. 10/526,049
Office Action Dated May 13, 2009

REMARKS

Favorable reconsideration is respectfully requested in view of the above amendments and following remarks. Claims 1, 7-12 and 15-16 have been amended editorially. Claim 4 is canceled without prejudice or disclaimer. Claims 17-19 are new. Claim 17 is independent, and tracks claims 1 and 4. Claims 18 and 19 track claims 5 and 6, respectively. No new matter has been added. Claims 1-3 and 5-19 are pending.

Claim rejections - 35 U.S.C. § 112

Claims 1-16 are rejected under 35 USC 112, first paragraph, as failing to comply with the enablement requirement. The rejection contends that the nature of the invention is directed to a method for preparing glucose dehydrogenase by transforming any microorganism with SEQ ID NO: 1, which comprises the nucleic acid coding region for the β subunit, wherein the glucose dehydrogenase does not comprise a β subunit. However, claim 1 recites introducing DNA containing SEQ ID NO: 1 into a particular microorganism, namely a microorganism belonging to the genus *Pseudomonas* to obtain the transformant. Thus, one skilled in the art would clearly understand that claim 1 requires a specific nucleotide sequence that is unique, and involves the expression of a unique sequence in a particular environment, that is, in a microorganism belonging to the genus *Pseudomonas*.

As explained in the specification, Applicants have found that the specific sequence SEQ ID NO: 1 is expressed differently in different environments. That is, when SEQ ID NO: 1 is expressed in *Burkohlderia cepatia* KS1 strain, the glucose dehydrogenase that is produced includes the α, β and γ subunits. However, when SEQ ID NO: 1 is expressed in *Escherichia coli*, the glucose dehydrogenase that is produced includes only the α and γ subunits. The glucose dehydrogenase that includes the α, β and γ submits is not produced (see page 2, line 15 to page 3, line 7). Even further, Applicants have found that when SEQ ID NO: 1 is expressed in a microorganism belonging to the genus *Pseudomonas*, two types of glucose dehydrogenases are produced: one that contains the β subunit and one that does not contain the β subunit. This is recited in claim 1. That is, claim 1 recites transforming a microorganism belonging to the genus *Pseudomonas* with SEQ ID NO: 1, and culturing the transformant to produce not only a second glucose dehydrogenase that does not contain the β subunit, but also a first glucose dehydrogenase that contains the β subunit.

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The rejection further contends that undue experimentation arises due to the unpredictability of forming a second glucose dehydrogenase without the β subunit, and steps to make a second enzyme without a β subunit while the coding sequence of the β subunit sequence is present is not clearly enabled. However, as indicated above, claim 1 recites culturing transformed *Pseudomonas* with SEQ ID NO: 1 to produce both the first glucose dehydrogenase that contains the β subunit and the second glucose dehydrogenase that does not contain the β subunit. Details are provided in the specification to permit one of ordinary skill in the art to obtain the two types of glucose dehydrogenase as recited in claim 1.

In particular, the Example begining on page 15 describes the preparation of SEQ ID NO: 1 for introduction into a microorganism belonging to the genus *Pseudomonas*. Specifically, chromosomal DNA from *Burkholderia cepatia* was isolated, and the SEQ ID NO: 1 was amplified. SEQ ID NO: 1 was then inserted into a vector, and the vector was introduced into *E. coli* and the *E. coli* was grown. A plasmid from the resulting *E. coli* colony was then used to transform a microorganism belonging to the genus *Pseudomonas*. The transformed microorganism belonging to the genus *Pseudomonas* was then cultured.

In order to isolate the glucose dehydrogenase from the cultured *Pseudomonas*, a crude enzyme solution was extracted from the cultured *Pseudomonas*, and the glucose dehydrogenase was eluted using a sepharose column by applying a NaCl gradient. The glucose dehydrogenase activity was measured for each fraction, and the results are shown in Figure 1. As shown in this figure, there were two major peaks where the NaCl gradient was applied. The fractions from the two major peaks were then collected, purified, and individually analyzed by running the purified fractions on an SDS-PAGE. The results are shown in Figure 2. As shown in Figure 2, the first fraction showed a band at 43 kDa, indicating that the glucose dehydrogenase of this fraction included the β subunit. The second fraction however did not show a band at 43 kDa, indicating that the glucose dehydrogenase of this fraction did not have the β subunit.

Thus, these experiments clearly show that a glucose dehydrogenases that includes the β subunit and a glucose dehydrognease that does not include the β subunit can be produced with a predictable result when a microorganism belonging to the genus *Pseudomonas* is transformed with SEQ ID NO: 1 and cultured, as recited in claim 1. Thus, contrary to the rejection's position, Applicants respectfully submit that the present specification would

clearly permit one of ordinary skill in the art to produce the first and second glucose dehydrogneases as recited in claim 1 without undue experimentation. Accordingly, claim 1 and its dependent claims comply with the enablement requirement.

As to claim 17, claim 17 further limits the species of *Pseudomonas*, namely *Pseudomonas putida*. This particular species was used in the Example described above to demonstrate that the two types of glucose dehydrogenase as claimed can be obtained with a predictable result. Thus, Applicants respectfully submit that no undue experimentation is required to make and use the invention of claim 17.

As to claim 5, claim 5 clarifies the source of SEQ ID NO: 1. As to claim 6, claim 6 identifies the exact source from which SEQ ID NO: 1 was obtained in the Example described above. Thus, Applicants respectfully submit that no undue experimentation is required to make and use the invention of claims 5 and 6.

Claims 7, 9 and 11 are rejected under 35 USC 112, first paragraph, as failing to comply with the written description requirement. Claims 7, 9 and 11 do not recite an amino acid sequence wherein one or a plurality of amino acid residues have been substituted, deleted, intercalated or added to the respective SEQ ID NO. Therefore, the rejection is rendered moot. Applicants do not concede the correctness of the rejection.

Claim Objections

Claims 1, 7-12, 15 and 16 are objected to because of informalities. Claims 1, 7-12, 15 and 16 have been amended, taking the issues noted in the objection into account. Withdrawal of the objection is requested.

In view of the above, favorable reconsideration in the form of a notice of allowance is requested. Any questions or concerns regarding this communication can be directed to the attorney-of-record, Douglas P. Mueller, Reg. No. 30,300, at (612) 455.3804.

Respectfully submitted,

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